Criteria Specification

ClinGen Cerebral Creatine Deficiency Syndromes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for SLC6A8 Version 1.1.0

Affiliation: Cerebral Creatine Deficiency Syndromes VCEP
Description : Cerebral Creatine Deficiency Syndromes Variant Curation Expert Panel ACMG Classification Rules Specified for Solute Carrier Family 6, Member 8 (SLC6A8; Creatine Transporter) Summary of ACMG-AMP Criteria for SLC6A8 Variants
Version : 1.1.0
Released : 9/14/2022
Release Notes :

- Added PM6. This was previously approved by SVI for Version 1.0 but had not been included in the CSpec Registry.
- Added clarification for PS2_Moderate.
- Added clarification for points system for PP4, all previously approved by SVI.

Rules for SLC6A8

Gene: SLC6A8 (HGNC:11055)
Preferred Transcript: NM_005629.4
HGNC Name: solute carrier family 6 member 8
Disease: creatine transporter deficiency (MONDO:0010305)

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.
Caveats:
• Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
• Use caution interpreting LOF variants at the extreme 3’ end of a gene.
• Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
• Use caution in the presence of multiple transcripts.

Very Strong

- Loss of function is a known mechanism of disease for Creatine Transporter Deficiency.
- Specifications are based on the decision tree as outlined in Tayoun etal, 2018 (Hum Mutat. 2018 Nov;39(11):1517-1524; PMID: 30192042) SLC6A8: PVS1, at appropriate strength, is applicable as described in Abou Tayoun et al, 2018 (PMID: 30192042)

Modification Type: None

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

PS1 is applicable as described.

Modification Type: None

PS2

Original ACMG Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Strong

Note: Confirmation of paternity in females only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

X-linked disorder. Only maternity needs to be confirmed.

Modification Type: Disease-specific,None

Moderate

Newly hemizygous male with the variant identified de novo in the mother with no family history of other affected males.

Modification Type: Disease-specific,Strength

PS3

Original ACMG
Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

RT-PCR evidence of mis-splicing for non-canonical intronic variants.

For non-canonical splicing variants, RT-PCR evidence demonstrating transcripts of alternative length or specific intron or exon inclusion/exclusion. These studies can be performed in patient derived cells, by placing the mutant genomic DNA into plasmid vectors, or by over-expressing mutant transcript. Assays should demonstrate defective splicing with RT-PCR analysis or RNA sequencing to confirm alternative transcripts.

Modification Type: Disease-specific

Supporting

- Creatine transport activity <10% wild type with less than or equal to 125uM creatine used in SLC6A8 deficient fibroblasts
- RT-PCR evidence of mis-splicing for non-canonical intronic variants with evidence of normal splice products. For non-canonical splicing variants, RT-PCR evidence demonstrating transcripts of alternative length or specific intron or exon inclusion/exclusion. These studies can be performed in patient derived cells, by placing the mutant genomic DNA into plasmid vectors, or by over-expressing mutant transcript. Assays should demonstrate defective splicing with RT-PCR analysis or RNA sequencing to confirm alternative transcripts.

Modification Type: Disease-specific

PS4

Original ACMG
Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.
Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Very Strong

- 4 independent male probands with elevated urine creatine/creatinine ratio on one occasion at minimum, in addition to any proband used for PP4.
- Variant must meet PM2_Supporting criterion for PS4 to apply.

Modification Type: Strength

Strong

- 3 independent male probands with elevated urine creatine/creatinine ratio on one occasion at minimum, in addition to any proband used for PP4.
- Variant must meet PM2_Supporting criterion for PS4 to apply.

Modification Type: Disease-specific

Moderate

- 2 independent male probands with elevated urine creatine/creatinine ratio on one occasion at minimum, in addition to any proband used for PP4.
- Variant must meet PM2_Supporting criterion for PS4 to apply.

Modification Type: Strength

Supporting

- One independent male proband in addition to any proband used for PP4.
- Variant must meet PM2_Supporting criterion for PS4 to apply.

Modification Type: Strength

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable
Comments: Not applicable

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing

Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Absent/rare from controls in an ethnically-matched cohort population sample. Threshold: <0.00002 (0.002%) AND 0 hemizygotes in gnomAD.

Modification Type: Disease-specific

PM3

Original ACMG Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Not Applicable

Comments: SLC6A8 is an X-linked gene, therefore PM3 is not applicable

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Moderate

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Modification Type: None

PM5

Original ACMG Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
Example: Arg156His is pathogenic; now you observe Arg156Cys.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Moderate

Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

Modification Type: None

Supporting

Missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before.

Modification Type: Strength

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Moderate

Variant identified as de novo in an affected male with the mother negative for the variant but maternity not confirmed.

Modification Type: No change

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
Note: May be used as stronger evidence with increasing segregation data.

Strong

- 3 affected segregations + 0 unaffected segregations OR
- 2 affected segregations + 3 unaffected segregations

Modification Type: Strength

Moderate

- 2 affected segregations + 0 unaffected segregations.

Modification Type: Strength

Supporting

- 1 affected family member + 3 unaffected segregations.

Modification Type: Disease-specific

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments: Not applicable, gnomAD (01/2019) expected missense 243.5, observed missense 117, for Z=2.94 (o/e =0.48). No constraint against missense variation.

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Supporting

- REVEL score >0.75 for missense variants
- In frame deletion or insertion predicted deleterious by PROVEAN, MutPred indel, and MutationTaster.
- Predicted impact on splicing by SpliceAI and varSEAK.

Modification Type: None

PP4

Original ACMG Summary

Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.

Strong

4 or more points based on combinations of the following.

- Elevated urine creatine/creatinine ratio on one occasion (1 point)
- Elevated urine creatine/creatinine ratio on more than one occasion (2 points)
- Significantly decreased creatine peak, with absent guanidinoacetate peak, if reported (3 points)
- Deficient creatine uptake in cultured fibroblasts (<10% of normal with <125uM creatine) (3 points)

Additional specifications:

- Two or more data types are required for PP4_Strong.
- An individual used to assign PP4, at any weight, cannot be also included for PS4 count. If multiple unrelated probands with the variant have been identified, it is recommended that the case with the highest PP4 points is assigned the appropriate weight for PP4, and the other cases are used for PS4.
- Variant must meet PM2_Supporting for PP4 to apply at any strength.
- For PP4 to be applied at strong, full SLC6A8 gene sequencing, including all coding exons and intron/exon boundaries, must have been carried out. If not, consider downgrading.

Modification Type: Disease-specific

Moderate

3 or more points based on:

- Elevated urine creatine/creatinine ratio on one occasion (1 point)
- Elevated urine creatine/creatinine ratio on more than one occasion (2 points)
- Significantly decreased creatine peak, with absent guanidinoacetate peak, if reported (3 points)
- Deficient creatine uptake in cultured fibroblasts (<10% of normal with <125uM creatine) (3 points)

Additional specifications:

- Two or more data types are recommended for PP4_Moderate.
- An individual used to assign PP4, at any weight, cannot be also included for PS4 count. If multiple unrelated probands with the variant have been identified, it is recommended that the case with the highest PP4 points is assigned the appropriate weight for PP4, and the other cases are used for PS4.
- Variant must meet PM2_Supporting for PP4 to apply at any strength.

Modification Type: Strength

Supporting

1-2 or more points based on:

- Elevated urine creatine/creatinine ratio on one occasion (1 point)

- Elevated urine creatine/creatinine ratio on more than one occasion (2 points)

Additional specifications:

- An individual used to assign PP4, at any weight, cannot be also included for PS4 count. If multiple unrelated probands with the variant have been identified, it is recommended that the case with the highest PP4 points is assigned the appropriate weight for PP4, and the other cases are used for PS4.
- Variant must meet PM2_Supporting for PP4 to apply at any strength.

Modification Type: Disease-specific

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

Allele frequency >0.0020 (0.2%) OR ≥10 hemizygotes in gnomAD

Modification Type: Disease-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Allele frequency > 0.0002 (0.02%) OR ≥ 5 hemizygotes in gnomAD

Modification Type: Disease-specific

BS2

Original ACMG

Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

Strong

Observed in the homozygous state in a healthy adult

Modification Type: None

BS3

Original ACMG

Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Supporting

- Creatine transport assay demonstrating ≥50% normal transport activity using physiological creatine concentrations (≤125μM creatine).
- RT-PCR evidence demonstrating no observable effect of splicing.
- Expression assay demonstrating wild type transcript levels

Modification Type: Disease-specific,Strength

BS4

Original ACMG

Summary

Lack of segregation in affected members of a family.
Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Strong

Lack of segregation in affected members of a family.

Modification Type: None

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

BP2

Original ACMG Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Not Applicable

BP3

Original ACMG Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

BP4

Original ACMG Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

- REVEL score <0.2 for missense variants
- In frame deletion or insertion predicted benign by PROVEAN, MutPred indel, and MutationTaster.
- No predicted impact on splicing by SpliceAI and varSEAK.

Modification Type: None

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Supporting

Variant found in a case with an alternate molecular basis for disease. BP5 applicable as described; the case must have specific features of creatine transporter deficiency, such as low creatine on brain magnetic resonance spectroscopy, or elevated urine creatine in order to apply this criterion. Presence of developmental delay and seizures is not sufficient.

Modification Type: None

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Modification Type: None

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS4_Very Strong)* **AND ≥ 1 Strong** *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)*

1 Very Strong *(PVS1, PS4_Very Strong)* **AND ≥ 2 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PS4_Very Strong)* **AND 1 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)* **AND 1 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1, PS4_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 2 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND ≥ 3 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND 2 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND 1 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Likely Pathogenic

1 Very Strong *(PVS1, PS4_Very Strong)* **AND 1 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1, PS4_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND 1 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

2 Moderate *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Moderate *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PS1, PS2, PS3, PS4, PP1_Strong, PP4_Strong)* **AND 2 Moderate** *(PS2_Moderate, PS4_Moderate, PM4, PM5, PM6, PP1_Moderate, PP4_Moderate)*

Benign

≥ 2 Strong *(BS1, BS2, BS4)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2, BS4)* **AND 1 Supporting** *(BS3_Supporting, BP4, BP5, BP7)*

≥ 2 Supporting *(BS3_Supporting, BP4, BP5, BP7)*